

REMARKS

I. Status of the Claims

Claims 1-30 are pending in the application, and claims 1-12, 23 and 28 stand withdrawn pursuant to a restriction requirement. Claims 13-22, 24-27, 29 and 30 have been examined and stand rejected, variously, under 35 U.S.C. §§101, 102 and 112 (first and second paragraphs). The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

Claims 13, 15, 20 and 29 are amended. Support for amendments to claim 15 can be found at page 11, line 32 to page 12, line 1, of the specification. Support for amendments relating to "stringent conditions" to each of claims 13, 15, 20 and 29 can be found at page 4, lines 14-17 of the specification.

II. Claim Objection

Claim 13 is objected to in the spelling of "homolog." An amendment is provided, thereby addressing the objection.

III. Rejections Under 35 U.S.C. §112

A. First Paragraph

Claim 15 is rejected as lacking written description and including new matter for the recitation inserted in the previous amendment. Applicants traverse, but have removed the language to which the examiner objects.

Claims 13-18, 20, 22, 25-27, 29 and 30 stand rejected as allegedly lacking an adequate written description under §112, first paragraph. The examiner has maintained the rejection on the grounds that the hybridization conditions recited are said to be "less" than the example

provided in the specification. Applicants traverse, but in the interest of advancing the prosecution, the claims have been amended to recite specific stringent hybridization conditions.

Reconsideration and withdrawal of both the foregoing rejections is respectfully requested.

B. Second Paragraph

Claims 13, 14, 16-18, 20, 22, 25-27, 29 and 30 are rejected as allegedly indefinite in the recitation of “stringent conditions.” Applicants traverse, but as discussed above, the claims have been amended to recite specific stringent conditions. Reconsideration and withdrawal of the rejection is respectfully requested.

IV. Rejections Under 35 U.S.C. §102

Claims 13-18, 20-22, 24-27, 29 and 30 remain rejected under §102(b) as allegedly anticipated by the ‘501 application. Applicants again traverse.

It is well known in the art that TT1 and TTG1 have distinct functions. Applicants direct the examiner to Haughn & Chaudhury, *Trends Plant Sci.* 10:472-477 (2005) (attached). The article shows that TT1 and TTG act in different regulatory pathways (see in particular p. 474, left col., last para. and right col. with Fig. 3). Their different functions are reflected by the fact that the comparison of TT1 and TTG1 amino acid sequences showed a **0% match!** This was noted in the previous response, and applicants now demonstrate by the attached comparison of coding sequences of TT1 and TTG1 (performed by DNA Strider 1.4f2 software) that there also is no substantial homology which would allow any fragment of TTG1 to hybridize to SEQ ID NO:2 or 4 (TT1) under the specified conditions. As such, the claims contain a recitation that is not met

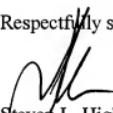
by the reference, and the subject matter of the present claims is cannot anticipated by the '501 application.

Reconsideration and withdrawal of the rejection is therefore respectfully requested.

VI. Conclusion

In light of the foregoing, applicants submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. Should the examiner have any questions or comments regarding this application, a telephone call to the undersigned is invited.

Respectfully submitted,



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Genetic analysis of seed coat development in *Arabidopsis*

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In the angiosperms, fertilization initiates the formation of the seed from the ovule, including the differentiation of the seed coat from the ovule integuments. Seed coat differentiation includes some of the most dramatic cellular changes of seed development and culminates in the death of the seed coat cells. Recently, genetic analyses in *Arabidopsis* have contributed substantially to our understanding of many aspects of seed coat biology and it might not be long before the entire differentiation pathway is understood. Such an advance would contribute substantially to our understanding of many important cellular events, including secondary cell wall synthesis, cell morphogenesis, vacuolar targeting and cell death, and would provide tools for the manipulation of seed dormancy and germination.

Seed coats

In the angiosperms, fertilization results in the formation of the seed from the ovule (Figure 1). This remarkable transformation involves the activation and coordination of the distinct developmental pathways leading to an embryo, endosperm and seed coat. The seed coat (testa) consists of several layers of specialized maternal cell types that provide an important interface between the embryo and the external environment during embryogenesis, dormancy and germination. Differentiation of the seed coat from the ovule integuments includes some of the most dramatic cellular changes observed during seed development and culminates in the death of the seed coat cells. Even in death, the specialized cell types impart protection, improve dormancy and germination, and enhance seed dispersal. In addition, the seed coat cells of some species, such as the seed coat epidermal trichomes of cotton (which we weave into textiles), are an important commercial commodity.

Relative to those of many other tissues of the seed, the molecular and cellular events underlying seed coat differentiation have received little attention. However, in recent years, the use of genetic analysis in *Arabidopsis* has contributed substantially to our understanding of many aspects of seed coat biology. In this article, we outline this progress, highlighting some of the interesting questions, including how seed coat development is initiated and coordinated with other seed tissues, what the structure and function of the various seed coat cell

layers are, and how the differentiation of the specialized cell types is regulated. Owing to space limitations, we do not discuss the genes required for ovule morphogenesis that also impact seed coat development but instead refer readers to reviews of the topic [1,2].

Overview of *Arabidopsis* seed coat development

In response to fertilization, the *Arabidopsis* seed coat differentiates primarily from cells of the ovule integuments (Figure 2a) over a period of 2–3 weeks [3–6]. Cells in both layers of the outer integument (Figure 2a, layers 1,2) and all three layers of the inner integument (Figure 2a, layers 3–5) go through a dramatic period of growth in the first few days after fertilization through both cell division and expansion (Figure 2b). The five cell layers follow one of four distinct fates (Figure 2c–e). Cells of the innermost layer (the endothelium; Figure 2a, layer 5) synthesize proanthocyanidin (PA) flavonoid compounds, also known as condensed tannins [7], which accumulate in the central vacuole during the first week after fertilization and later oxidize, imparting a brown color to the seed coat. By contrast, cells of the other two inner integument layers (Figure 2a, layers 3,4) do not appear to differentiate further and are crushed together as the seed develops (Figure 2d,e). Cells of both outer integument layers (Figure 2a, layers 1,2) accumulate starch-containing

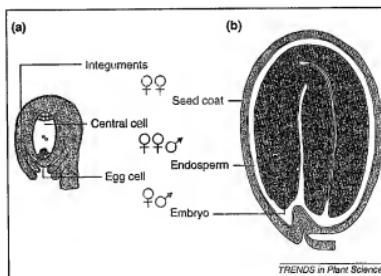


Figure 1. The seed is derived from the ovule (a). The egg and sperm nuclei fuse to form the diploid zygote that develops into the embryo (b). A second sperm cell fuses with the triploid central cell, initiating triploid endosperm development (b). These fertilization events induce the maternal diploid integument cells to differentiate into a seed coat (b).

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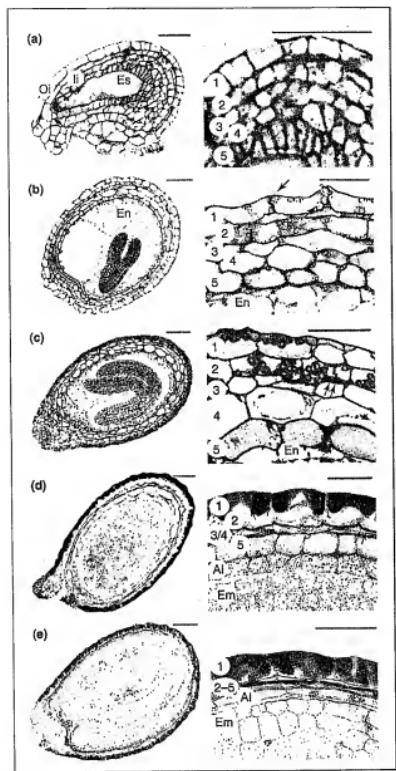


Figure 2. Development of the ovule integuments into a seed coat following fertilization. This figure shows several stages (a-e) of seed development for the whole seed (left) and a detail of the developing seed coat (right). The two cell layers of the ovule outer integument (a), (b), (c) and three cell layers of the inner integument (a), (b), (c-d) undergo a period of growth within the first 5 days after fertilization (b). Cells of individual layers undergo a series of cell divisions, increasing the thickness of the epidermis (2) and epidermis (1), a process that is almost complete 10 days after fertilization (d). By seed maturity (15 days) (e), cells of all layers are dead and have been crushed together, except for the epidermis, the shape of which is maintained by the thick secondary cell wall of the columella. Single arrows indicate starch-containing plastids (b), mucilage in the apoplast (c) or secondary cell wall forming in the epidermis (d). The double arrows indicate the secondary cell wall of the palisade (c-d). Abbreviations: Al, endosperm aleurone; Em, embryo; En, endosperm; Es, embryo sac; II, inner integument; OI, outer integument. Scale bar = 80 μ m (b-d, left); 40 μ m (a, left, a-d, right).

amyloplasts during the growth phase (Figure 2b) before diverging in fate. The subepidermal layer (Figure 2a, layer 2) produces a thickened wall on the inner tangential side of the cell (palisade, Figure 2c-e). The cells of the

epidermal layer (Figure 2a, layer 1) synthesize and secrete a large quantity of mucilage (a pectinaceous carbohydrate) into the apoplast specifically at the junction of the outer tangential and radial cell walls (Figure 2c). As mucilage deposition proceeds, the vacuole contracts, leaving a cytoplasmic column in the center of the cell surrounded by a donut-shaped apoplastic space filled with mucilage. Following mucilage synthesis, a secondary cell wall is deposited that completely fills the space occupied by the cytoplasmic column, forming the columella (Figure 2d,e).

During the later stages of seed development, the cells of all seed coat layers die. The structure of the epidermal cells is preserved by the mucilage and columella, and the remaining layers are crushed together by the end of seed maturation. PAs are apparently released from the endothelial cells and impregnate the inner three cell layers during this period (Figure 2e).

Interactions between seed coat and endosperm during early seed coat development

Seed coat growth and differentiation are initiated by fertilization and proceed coordinately with those of the embryo and endosperm [3-5]. Unlike the embryo and endosperm, the seed coat is not directly involved in the fertilization process. Therefore, one or more events during or following fertilization must signal the seed coat to develop coordinately with the other tissues. Two recent studies have used mutants to provide evidence for the influence of the endosperm on both the growth and the differentiation of the seed coat.

The control of seed coat growth relative to that of the embryo and endosperm appears to be complex [8,9]. Seed coat growth occurs soon after fertilization and involves both cell division and cell elongation (Figure 2b). Limitations in seed coat cell division are compensated for by an increase in cell elongation, but the converse is not true. This indicates that cell elongation, but not division, responds to seed size. Mutations in the *HAIKU* gene result in limited growth of the syncytial endosperm [9]. This defect in endosperm growth also has a non-cell-autonomous effect on growth of the developing seed coat such that cell elongation (but not cell division) in the expanding seed coat is restricted. These results suggest that the growing endosperm regulates the extent to which the ovule integument cells elongate following the initiation of seed coat development. Conversely, loss of function mutations in the *TRANSPARENT TESTA GLABRA2* (*TTG2*) gene [10] restrict cell elongation of the seed coat, which limits endosperm growth in a non-cell-autonomous manner [8]. This 'cross-talk' between the developing endosperm and seed coat appears to coordinate growth between the endosperm and seed coat, ultimately establishing seed size. Although the mechanism for the coordinate control of growth is not known, mechanical force or restraint is consistent with all data, but endosperm-seed-coat signaling cannot be ruled out.

Evidence for the involvement of the endosperm in the initiation of seed coat development has come from the analysis of seed coat development (G. Haughn *et al.*, unpublished) in sporophytic (*titan1* [11]) and gametophytic (*fertilization independent seed development1-fertilization*

independent seed development3 [12]) lethal mutants. These studies suggest that a signal from the syncytial endosperm is sufficient to initiate seed coat development in cells of the ovule integument. Once initiated, all aspects of seed coat differentiation, except growth (see below), appear to proceed independently of both endosperm and embryo development. The nature of the signal and the mechanism through which it is enacted are key questions that still need to be answered.

Endothelial development

The isolation of mutants with altered seed coat color (*transparent testa* (*tt*), *transparent testa glabra* (*ttg*) and *banyuls* (*ban*) [7,13,14]) or PA levels (*tannin deficient seed* (*tds*) [15]) have enabled the identification of genes required for the differentiation of the flavonoid-producing endothelial cells. Many of these genes have been cloned and can be grouped into two general classes based on the function of their products. One class of genes encodes proteins required for the biosynthesis and compartmentation of the flavonoid compounds; these include *TT3*, *TT4*, *TT5*, *TT6*, *TT7*, *BAN*, *TT12*, *TT19*, *TD54/TT18* and *AUTOINHIBITED H⁺-ATPase ISOFORM 10 (AHA10)* [7,14–18]. These genes have been useful in elucidating the PA biosynthetic pathway. A notable example was the discovery of the *ban* mutant, which accumulates anthocyanin instead of PAs in endothelial cells [19]. The cloning of *BAN* and the characterization of its product identified a previously unknown but pivotal enzymatic step, anthocyanidin reductase, in the PA biosynthetic pathway [20–22]. Another important aspect of PA biosynthesis concerns the targeting of the precursors to the vacuole. The genes *TT12*, *TT19* and *AHA10* encode a putative multidrug and toxic compound extrusion transporter, glutathione-S-transferase and H⁺-ATPase, respectively, and are believed to be involved in transporting flavan-3-ols (PA precursors) to the vacuole. This conclusion is based on the deduced identity of the gene products and the phenotypes of the loss of function mutants, which include a late block in PA biosynthesis (*tt12*, *tt19* and *aha10*) and disruption in vacuolar biogenesis in endothelial cells (*tt12* and *aha10*) [17,18,23]. It is possible that one or more flavonoid compounds produced by the PA pathway are bound by glutathione-S-transferase in the cytoplasm and transported to the vacuole via a vacuolar protein pump that requires H⁺-ATPase activity. However, considerably more work is required to substantiate and characterize this putative transport process.

The second class of TT genes encodes transcription factors (*TT1*, *TT2*, *TT8*, *TT16*, *TTG1*, *TTG2*). *TT2* is an endothelial-specific regulatory protein (a MYB transcription factor) that is necessary for PA biosynthesis. In addition to controlling PA biosynthesis in the endothelium, *TT8*, basic helix-loop-helix (bHLH) transcription factor, also functions in the seed coat epidermis (see below). Both proteins act, at least in part, by directly regulating transcription of flavonoid biosynthetic genes, such as *TT3* and *BAN*, 3–4 days after fertilization (late globular stage of embryogenesis [6,16,24–26]). *TTG1*, a WD40 repeat protein [27], and *TTG2*, a WRKY protein [10], have roles in endothelial cells similar to those of *TT8*

and *TT2* but, in addition, are required for the differentiation of a range of other cell types in the plant, including trichomes, root hairs and seed coat epidermal cells [10,28–31] (see below). *TTG1* forms an endothelium-specific transcription complex with *TT8* and *TT2* (Figure 3c) [26] to activate transcription of the target genes. This complex is analogous to those *TTG1* form with different bHLH and MYB polypeptides to specify the differentiation of several other plant cell types [32,33]. By contrast, *TTG2* must function downstream of the *TTG1* complex because its transcription is dependent on *TTG1* activity (Figure 3) [10]. However, *TTG2* is not required for *BAN* transcription [6] and so it must play a different role in PA biosynthesis. Two other transcription factors, *TT1* (a WIP zinc-finger protein [34]) and *TT16* (a MADS protein [35]), are produced specifically in the endothelium and are required for PA biosynthesis. The probable role of *TT16* is in specifying endothelial cell type because it is required not only for normal transcript levels of *ban* but also for normal endothelial cell shape and vacuolization. Indeed, cells derived from all three layers of the inner integument appear to

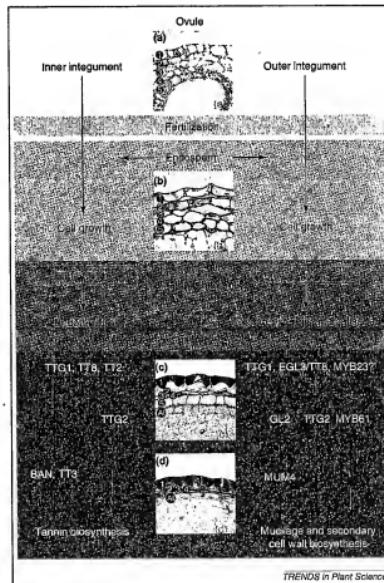


Figure 3. Seed coat differentiation is controlled by a series of regulatory steps beginning with fertilization of the ovule (a). The developing endosperm influences integument development into the seed coat (b) and layer-specific regulatory cascades lead to differentiation (c-d), including an increase in the transcription of genes encoding enzymes needed for PA and mucilage biosynthesis. 1–6 in (a–d) indicate the cell layers of the seed coat. Abbreviation: AI, aleurone cells.

divide and differentiate abnormally in the *tt16* mutant, suggesting that TT16 controls the differentiation of all cells of the inner integument [6,35]. Thus, TT16 can be considered to be a global regulator of endothelial differentiation, lying upstream of the other transcription factors (Figure 3). The specific role of TT1 is less clear. TT1 is not required for *ban* transcription [6] and there have been conflicting reports concerning its role in establishing endothelial cell shape [6,34]. TT1 could promote aspects of endothelial differentiation required for PA biosynthesis that are independent of the TT2-TT8-TTG1 complex and its targets. Determinating the expression of *tt1* and of *tt2*, *tt6*, *ttg1*, *ttg2* and *tt16* in the *tt1* mutant background might shed some light on the role of TT1. It is interesting that TT1 and TT16 are not required for the differentiation of a small section of seed coat endothelium in the chalazal and micropylar ends of the seed. The differentiation of such cells must be under the control of a separate regulator(s).

Seed coat epidermal development

Mutants defective in seed coat epidermal development have been identified primarily through a decrease in the amount or chemical composition of the seed coat mucilage extruded when the seed is exposed to water [mucilage modified (*num1*), *ttg*, *myb* protein 61 (*myb61*), *apetala2* (*ap2*), *glabrat2* (*gl2*), *enhancer of glabrat3* (*egl3*) and *tt8*]. As in the case of endothelial differentiation, the epidermal mutants define genes that fall into two general classes: those apparently required for mucilage biosynthesis and those regulating differentiation.

Mutations in five genes result in seed coats with an altered amount (*num4*) or composition (*num1*, *num2*, *num3*, *num5*) of mucilage [31,36]. Mucilage is composed primarily of pectin, the major component of which is rhamnogalacturonan 1 (RG1) [4,36-39]. Because little is known about the biosynthesis of this important carbohydrate, the MUM genes represent a valuable resource. Three of these genes have been cloned. Mutations in *num4* result in a significant decrease in seed coat mucilage. *num4* encodes an enzyme thought to be required for the biosynthesis of rhamnose, a monosaccharide that is in high abundance in mucilage [36,40]. *num4* transcript is upregulated in the seed coat during the period when mucilage is synthesized. Interestingly, MUM4 is required not only for normal levels of mucilage but also for complete formation of the columella and contraction of the vacuole. These data suggest that normal mucilage biosynthesis has a role in cell morphogenesis, an intriguing connection that needs to be investigated. *num2*, the loss of function phenotype of which includes defects in the extrusion of mucilage from the seed coat, has recently been shown to encode a glycosyl hydrolase (G. Dean *et al.*, unpublished). Mutations in *num5* result in mucilage with altered cohesive properties. *num5* encodes a pectin methyl esterase (M. Facette and C.R. Somerville, unpublished). More extensive biochemical and molecular analyses of these genes, their products and their roles might shed light on the biosynthesis and biological properties of pectinaceous compounds.

Seven additional genes required for normal differentiation of the seed coat epidermis encode transcription

factors (*ttg1*, *ttg2*, *myb61*, *ap2*, *gl2*, *egl3* and *tt8*). A TTG1 regulatory complex, analogous to the one that controls trichome and root hair differentiation and PA biosynthesis in the endothelium [6,32], regulates the biosynthesis of mucilage during seed coat epidermal differentiation. EGL3 is a bHLH transcription factor that, like TT8, interacts with TTG1 and is partially redundant with TT8 in promoting mucilage biosynthesis [41]. Thus, both EGL3 and TT8 act as the TTG1 complex bHLH transcription factor that is involved in epidermal differentiation.

To date, no MYB protein has been clearly identified that acts in a complex with TTG1 and EGL3/TT8. One possible candidate is MYB23 because it is closely related in sequence to other MYB proteins that are known to act with TTG1 (GL1, WER and TT2) (Figure 3) [32,42-44]. In addition, transgenic *Arabidopsis* plants that show ectopic production of a chimeric MYB23 fused to a transcriptional repressor domain from the SUPERMAN protein lacks seed coat mucilage, suggesting that MYB23 could have a role in seed coat epidermal development [45,46]. Because loss-of-function mutations in *myb23* have no phenotype and expression in the seed coat has not been demonstrated, strong conclusions concerning the role of *myb23* in seed coat development cannot be made. Another MYB gene known to be required for seed coat epidermal differentiation is *myb61* [38]. However, MYB61 is not a good candidate for membership of a TTG1 complex because it belongs to a MYB subfamily that is only distantly related to GL1/WER and preliminary evidence suggests that TTG1 and MYB61 do not regulate the same targets in the seed coat epidermis (see below).

The transcription factors GL2 and TTG2 act downstream of TTG1 (Figure 3) [10,47,48]. However, despite the similarity in their loss of function phenotypes in the seed coat epidermis, the downstream targets of the two transcription factors do not appear to be identical (see below). Based on loss of function phenotypes, TTG1, GL2, MYB61 and TTG2 are all required for both mucilage synthesis and columella formation. Expression analyses have shown that the upregulation of *num4* requires both TTG1 and GL2 but not MYB61 or TTG2 [36]. These data suggest that MYB61 and TTG2 control different aspects of mucilage biosynthesis from TTG1/GL2 (Figure 3). Targets of MYB61 and TTG2 remain to be identified.

AP2 is distinct from the other regulators of the seed coat epidermis in that it apparently acts earlier and affects a wider array of seed coat epidermal characteristics. Functional AP2 is required for all aspects of differentiation of both the epidermis and palisade layers following growth of the integument cells [31,49]. Consistent with this hypothesis is the fact that AP2 is required for normal levels of GL2, TTG2 and MUM4 transcript in the seed coat [36]. Thus, AP2 could be the outer integument counterpart of TT16 in being an early regulator of cell type (Figure 3).

Programmed cell death in the seed coat

By the time the seed coat is mature, the cells in all the layers are dead. The various layers die at different times of development and in a specific sequence, suggesting that the cell death is programmed as part of the differentiation process. The cell layers that undergo cell death earliest are

the two outer layers of the inner integument, which do not appear to develop specialized features (Figure 2, seed coat layers 3 and 4). A proteinase with caspase-like activity and sequence similarity to a family of vacuolar processing enzymes (VPEs), termed δ VPE, was found to appear specifically in seed coat layers 3–4 early in seed coat development (maximum at 4–5 days after fertilization) [50] and this production of δ VPE is correlated with programmed cell death (PCD) in these layers. Indeed, loss of function mutations in the gene encoding δ VPE significantly delayed the onset of PCD. Questions about additional proteins involved in PCD, the manner in which PCD is regulated and whether similar processes promote cell death in the other layers remain to be addressed.

Functions of the seed coat

The roles typically ascribed to the seed coat include the promotion of dormancy, protection and dispersal. In the *Arabidopsis* seed coat, the special features attained through differentiation include inner cell walls impregnated with PA, two sets of thickened secondary cell walls in the outer two layers, and mucilage in the apoplast of the epidermis that is extruded upon exposure to water. In other species, the presence of PA has been associated with resistance to pathogens and herbivores. Secondary cell walls are assumed to provide support, protection and impermeability to water and oxygen. It has been proposed that mucilage assists germination and protects the seed against toxic chemicals. The availability of mutants defective in the seed coat epidermis, palisade and endothelium has been useful in testing some of these hypotheses concerning seed coat function. Most seed coat mutants tested, including those specifically defective in PA biosynthesis (e.g. *ban*) and the secondary cell walls of the outer layers (e.g. *ap2*), but not those specifically defective in mucilage biosynthesis (e.g. *gl2*), had seed coats that were significantly more permeable than those of the wild type. In addition, most mutants produced seeds that were less dormant and showed a decreased capacity for germination following long-term storage. Furthermore, several mutants (*tt14*, *tt12* and *ttg1*) germinate more readily in response to gibberellin acid treatments [28,51,52]. These data support the hypothesis that one role of PA, thick secondary cell walls and mucilage is to restrict the exchange of gases and fluids between the environment and the seed, prolonging the life of the dormant embryo as well as protecting it and preventing germination until conditions are favorable. Mutants with reduced mucilage also have a decreased ability (relative to the wild type) to germinate under conditions of limited water availability [38], suggesting that hydrated mucilage provides an aqueous environment that assists germination.

Future prospects

Seed coat development involves a fascinating interaction between endosperm and maternal tissue, and several unique differentiation pathways ending in cell death. The dispensable nature of the seed coat and its accessibility make it a good model genetic system for cell differentiation, and have enabled researchers to identify a regulatory cascade for two cell types, to define the biosynthetic

pathway of PA and to verify its role in seed dormancy, germination and storage longevity. Further analysis of the seed coat has considerable potential to make additional significant contributions to our understanding of cell biology. First, the manner by which the endosperm and seed coat communicate has yet to be determined. Second, because all the integument cells originate from the first (epidermal) layer, it is unclear how the different cell types in the seed coat are determined. The fact that ablation of the endothelial layer did not influence adjacent cells of the inner integument to differentiate as endothelial cells [6] suggests that layer determination might not be based primarily on position, although it is possible that early cell-cell inductive processes occurred before ablation. Third, the mechanisms for PA deposition in the vacuole await investigation; the possibility of defining both regulatory and enzymatic steps in pectin and secondary cell wall biosynthesis holds promise. Fourth, mucilage is secreted to a specific domain of the apoplast (at the junction of the outer tangential and radial cell walls), resulting in a donut-shaped apoplast and cytoplasmic column. Thus, studying the seed coat epidermis could help to elucidate targeted secretion and cell morphogenesis in plants. Finally, the seed coat is a powerful model system for studying PCD. These, and many other questions that will arise in the future, should keep cell and developmental biologists interested in the seed coat for many years to come.

Acknowledgements

We apologize to those whose research we do not cite because of space limitations. We thank Tamara Western for the conceptual design of Figure 1 and Tamara Western, Sharon Abrahams and Gillian Dean for comments on the manuscript. G.H. was supported by Killam and MacMaster fellowships while on sabbatical leave at CSIRO Plant Industry and an NSERC Discovery Grant for his research in Canada.

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Method: Blocks (Martinez)
Layout: Standard
Mismatch penalty: Smaller (1)
Gap penalty: Medium (2)
Translation: Off

% Identity = 3.9 (72/1836)